CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, June 2005, p. 797–800 Vol. 12, No. 6
1071-412X/05/$08.00+0 doi:10.1128/CDLI.12.6.797–800.2005

Surface Envelope Glycoprotein Is B-Lymphocyte Immunodominant in Sheep Naturally Infected with Ovine Progressive Pneumonia Virus

Lynn M. Herrmann,[1,2]* Travis C. McGuire,[2] Isidro Hötzel,[2] Gregory S. Lewis,[3] and Donald P. Knowles[1,2]

Animal Disease Research Unit, Agricultural Research Service, U.S. Department of Agriculture, Pullman, Washington 99164-6630[1]; Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, Washington 99164-7040[2]; and U.S. Sheep Experiment Station, Agricultural Research Service, U.S. Department of Agriculture, Dubois, Idaho 83423[3]

Received 10 November 2004/Returned for modification 10 March 2005/Accepted 18 March 2005

The B-lymphocyte-immunodominant antigen involved in naturally ovine progressive pneumonia virus (OPPV)-infected mature sheep remains unknown. Therefore, the amount of antibody in sera from 10 naturally OPPV-infected sheep was evaluated by immunoprecipitation (IP) of the major viral proteins in [^{35}S]methionine/cysteine-labeled OPPV (whole virus) lysate. Using an excess of OPPV proteins in whole-virus lysate, 8 out of 10 sheep had the highest serum antibody IP endpoint titers to the gp135 surface envelope glycoprotein (SU). Also, 2 out of 10 sheep had equivalent serum antibody IP endpoint titers to the transmembrane glycoprotein oligomer (TM90) and SU. Since these data indicate that SU is the immunodominant protein in most mature sheep persistently infected with OPPV, SU-specific diagnostic serological assays can be utilized for OPPV diagnosis.

Ovine progressive pneumonia virus (OPPV) is a small ruminant lentivirus (SRLV) that can cause severe dypsnea in some flocks, eventually leading to death. OPPV is considered the North American equivalent to maedi-visna virus (MVV) that caused 20 to 30% mortality in Icelandic flocks in the late 1930s (10). However, most sheep live a normal life despite the presence of persistent OPPV infection as diagnosed by the presence of anti-OPPV antibodies in serum. Although antibodies are detected, the B-lymphocyte immunodominant antigen in naturally OPPV-infected mature sheep remains unknown. B-lymphocyte immunodominance in persistent viral infections is defined as greater antibody production to one viral antigen compared to other viral antigens. Therefore, in this study, the amount of anti-OPPV antibody in sera from 10 6-year-old naturally OPPV-infected sheep was evaluated by titration in immunoprecipitation (IP) with major viral proteins in [^{35}S]methionine/cysteine-labeled OPPV (whole virus) lysate.

The 10 sheep used in this study were defined as OPPV-infected based on the presence of serum antibodies that inhibited monoclonal antibody (MAb) 74A binding to caprine arthritis encephalitis virus (CAEV) surface envelope glycoprotein (SU) in a competitive inhibition enzyme-linked immunosorbent assay (cELISA) (VMRD, Inc., Pullman, WA). CAEV cELISA titers were determined by endpoint serial dilution of serum into 1× phosphate-buffered saline (PBS), pH 7.0. [^{35}S]methionine/cysteine-labeled OPPV WLC1 lysates were prepared using previously established methods with slight modifications (3, 4). Briefly, neonatal goat synovial membrane (GSM) cells isolated from CAEV-negative kids were grown at 37°C in 5% CO_2 in minimal essential medium (MEM) (Invitrogen) supplemented with 2% fetal bovine serum (FBS) and were infected at a multiplicity of infection (MOI) of 0.5 with OPPV WLC1. Typically, cytopathic effects were observed in the GSM cells within 7 to 8 days after OPPV WLC1 infection and media was removed and replaced with methionine-free MEM (Invitrogen) for 4 h. Following this, 0.5 to 1.0 mCi of NEG-772 Easytag Express Protein Labeling Mix [^{35}S] containing L-[^{35}S]methionine and L-[^{35}S]cysteine (Perkin Elmer) was added to the methionine-starved, OPPV WLC1-infected GSM cells in a 800-cm^2 roller bottle. After 2 to 4 h, FBS was added to a final concentration of 2%, and the cultures were incubated at 37°C at 5% CO_2 for 5 to 6 days. The supernatant was collected and centrifuged at 1,400 × *g* for 15 min at 4°C. The virus-containing supernatant was ultracentrifuged at 131,000 × *g* for 90 min at 4°C, and the resulting viral pellet was resuspended into 1× cell lysis buffer (CLB) composed of 1× Tris-saline-EDTA (TNE), pH 7.4, with 1% NP-40, 0.5% deoxycholate, and 0.1% sodium dodecyl sulfate on ice. The viral lysate was clarified by ultracentrifugation at 183,000 × *g* for 60 min at 4°C, and the resulting viral lysate supernatant passed through a 0.22-μm filter. Five microliters of the filtered [^{35}S]methionine/cysteine-labeled OPPV WLC1 lysate was counted by precipitating viral proteins using trichloroacetic acid (TCA) followed by liquid scintillation. The [^{35}S]methionine/cysteine-labeled OPPV WLC1 lysate was stored at −20°C until use.

In order to identify the OPPV protein that induced an immunodominant B-lymphocyte response, it was determined that the major OPPV proteins p28 capsid protein (CA), transmembrane glycoprotein monomer (TM38), transmembrane glycoprotein oligomer (TM90), and gp135 surface envelope glycoprotein (SU) were present and in excess by titrating [^{35}S] methionine/cysteine-labeled OPPV WLC1 lysate in an immunoprecipitation reaction while keeping the amount of sera

* Corresponding author. Mailing address: 3003 ADBF, Washington State University, Pullman, WA 99164-6630. Phone: (509) 335-6068. Fax: (509) 335-8328. E-mail: lherrman@vetmed.wsu.edu.

constant. Immunoprecipitation reactions were conducted using previous methods, except where indicated (3, 4). An OPPV WLC1 lysate titration was performed by mixing 10 μl of serum from each of the 10 OPPV-positive ewes (LMH 11-20) with 1 $\times 10^6$, 5×10^5, 1×10^5, 5×10^4, 1×10^4, 5×10^3, or 1×10^3 counts per minute (cpm) of [^{35}S]methionine/cysteine-labeled OPPV WLC1 lysate in an immunoprecipitation reaction. The total IP reaction volume was 200 μl in 1× CLB. At 5×10^5 cpm of [^{35}S]methionine/cysteine-labeled OPPV WLC1 lysate, CA, TM38, TM90, and SU were in excess (see arrows in Fig. 1A, lane 5, and B, lane 5).

To determine the immunodominant B-lymphocyte OPPV antigen, serum was serially diluted 1:2, 1:4, 1:8, 1:16, 1:32, and 1:64 in the immunoprecipitation reaction, keeping 5×10^5 cpm of [^{35}S]methionine/cysteine-labeled OPPV WLC1 lysate constant and the total IP reaction volume at 200 μl in 1× CLB buffer. Two-week exposed autoradiographs were analyzed using an Alphaimager (Alpha Innotech Corp., San Leandro, CA) using software provided by the imager. Adjusted signal peak areas were determined by measuring the signal peak areas for the immunoprecipitated OPPV proteins: CA, TM38, TM90, and SU at each dilution minus the background signal peak areas corresponding to where CA, TM38, TM90, and SU would migrate in the negative control serum (undiluted). The adjusted signal peak areas were determined within the linear range (nonsaturating) of signal on the Alphaimager. The adjusted signal peak area (y axis) for each OPPV protein was plotted against the inverse serum dilution (x axis), and the resulting x intercept ($y = 0$) from the linear regression was defined as the IP endpoint titer for that OPPV protein. Representative results from two serum titrations are shown where IP endpoint titers were highest against SU (Fig. 1C, lanes 5 to 8, and D, lanes 5 to 9). IP endpoint titers were highest for SU in 8 out of 10 OPPV-infected sheep and equally high for SU and TM90 in 2 out of 10 OPPV-infected sheep (Fig. 2). In all 10 OPPV-infected sheep, serum IP antibody endpoint titers were lower for CA and TM38 than SU or TM90.

The results of this study indicate that SU is the immunodominant B-lymphocyte OPPV protein in most mature sheep (6 years old) naturally infected with OPPV. Interestingly, another study showed that anti-CA antibodies were first to be detected by Western blot analysis during natural and experimental maedi-visna infection in sheep (5). These studies together suggest that the B-lymphocyte-immunodominant viral antigen may change during OPPV infection. This is a possibility given that the B-lymphocyte-immunodominant OPPV antigen was CA 3 years prior in only one sheep (CA endpoint titer, 8; SU endpoint titer, 4) out of seven in our study (LMH 11) by immunoprecipitation of [^{35}S]methionine/cysteine-labeled OPPV WLC1 lysate (data not shown).

The fact that SU is immunodominant in most OPPV-infected sheep provides a basis for the observed high sensitivity and specificity of a caprine arthritis-encephalitis virus (CAEV) competitive enzyme-linked immunosorbent assay (cELISA) in detecting anti-SU antibodies in serum from OPPV-infected sheep ranging in ages 3 to 7 (4). The CAEV cELISA relies upon the presence of anti-SU antibodies to inhibit the binding of monoclonal antibody 74A to CAEV-63 SU (9). Interestingly, cELISA endpoint titers and IP endpoint titers do not correlate within the same animal in some instances (see LMH 16, LMH 17, and LMH 20 in Fig. 3). This indicates that in some sera the two assays are measuring different anti-SU antibodies.

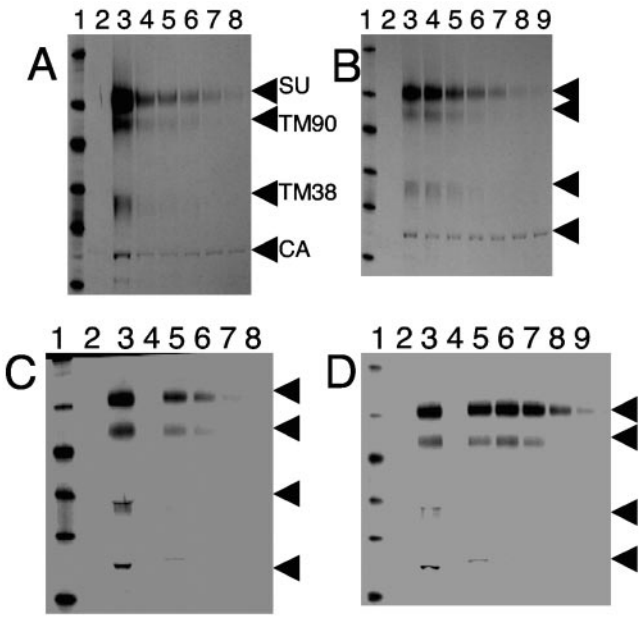

FIG. 1. Representative immunoprecipitations (IP) of [^{35}S]methionine/cysteine-labeled OPPV WLC1 lysate using sera from two naturally OPPV-infected mature ewes. Panels A and C represent sheep LMH 12, which had overall low serum antibody titers to OPPV proteins, and panels B and D represent sheep LMH 15, which had overall high serum antibody titers to OPPV proteins. The arrowheads on the right of panels A to D designate the OPPV proteins SU, TM90, TM38, and CA. Panels A and B show that the OPPV proteins SU, TM90, TM38, and CA are in excess at 5×10^5 ^{35}S counts per minute (cpm)/IP reaction while keeping the amount of serum constant (lane 5). For panels A and B, lane 1 shows the apparent molecular masses of ^{14}C-labeled protein markers at 200 kDa, 92.5 kDa, 69 kDa, 46 kDa, 30 kDa, and 14.3 kDa. Lanes 2 and 3 are negative and positive control sera using 1×10^6 ^{35}S cpm/IP reaction. Lanes 4 to 9 show the IP results by diluting the [^{35}S]methionine/cysteine-labeled OPPV WLC1 lysate in serial dilutions, starting with 1×10^6 counts/IP reaction, while holding the amount of sera constant. Panels C and D show that sheep LMH 12 and LMH 15 had the highest amount of anti-OPPV antibody directed toward SU in regards to IP and are representative for 8 out of 10 sheep. For panels C and D, lane 1 shows the apparent molecular mass of ^{14}C-labeled protein markers. Lanes 2 and 3 are negative and positive control sera using 5×10^5 ^{35}S cpm/IP reaction. Lane 4 is empty. Lanes 5 to 9 show the IP results when sera are serial diluted while holding the [^{35}S]methionine/cysteine-labeled OPPV WLC1 lysate constant at 5×10^5 cpm/IP reaction.

Our study was the first to verify that the OPPV proteins CA, TM38, TM90, and SU were present and in excess prior to titrating the sera, leading to a more accurate determination of SU as the immunodominant B-lymphocyte OPPV antigen. However, specific activity (Ci/mmol) or the number of ^{35}S's incorporated into the methionines and cysteines of SU, TM90, and CA could also explain our results. Theoretically, SU contains more ^{35}S-labeled methionines/cysteines per molecule (37 total in OPPV WLC1) than TM90 (30 total in OPPV 85/34) or CA (12 total in OPPV WLC1). So, if SU IP endpoint titers were three times more than CA and TM90 IP endpoint titers were 2.5 times more than CA, specific activity may be playing a role in determining the B-lymphocyte-immunodominant

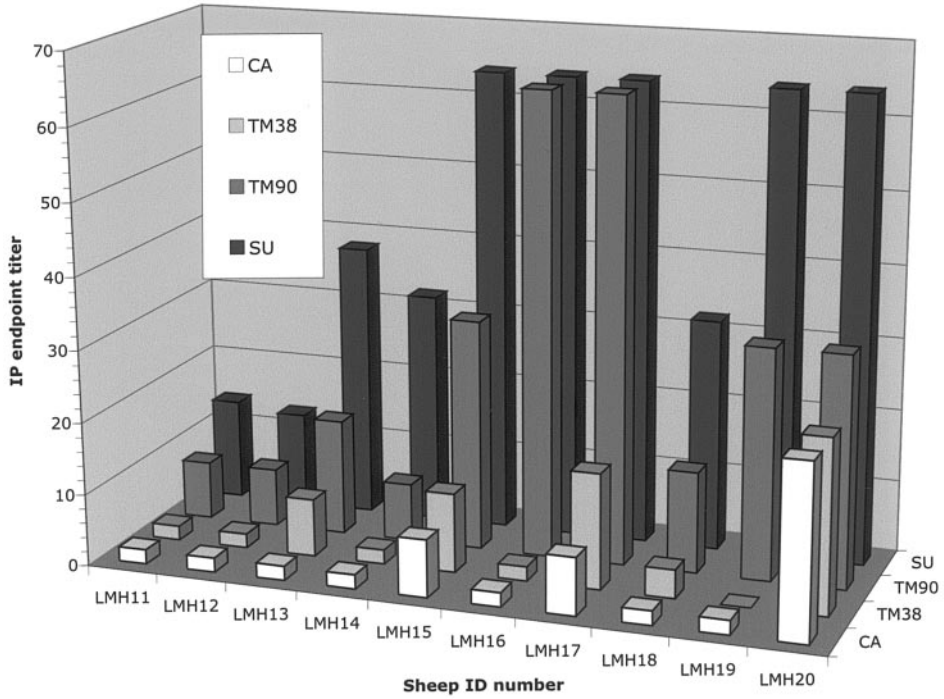

FIG. 2. SU is immunodominant in 8 out of 10 naturally OPPV-infected sheep (LMH 11, 12, 13, 14, 15, 18, 19, and 20), and both SU and TM90 are immunodominant in 2 out of 10 naturally OPPV-infected sheep (LMH 16 and 17). IP endpoint titers were calculated (see the text) for each ewe for following OPPV proteins: CA, TM38, TM90, and SU. ID, identity.

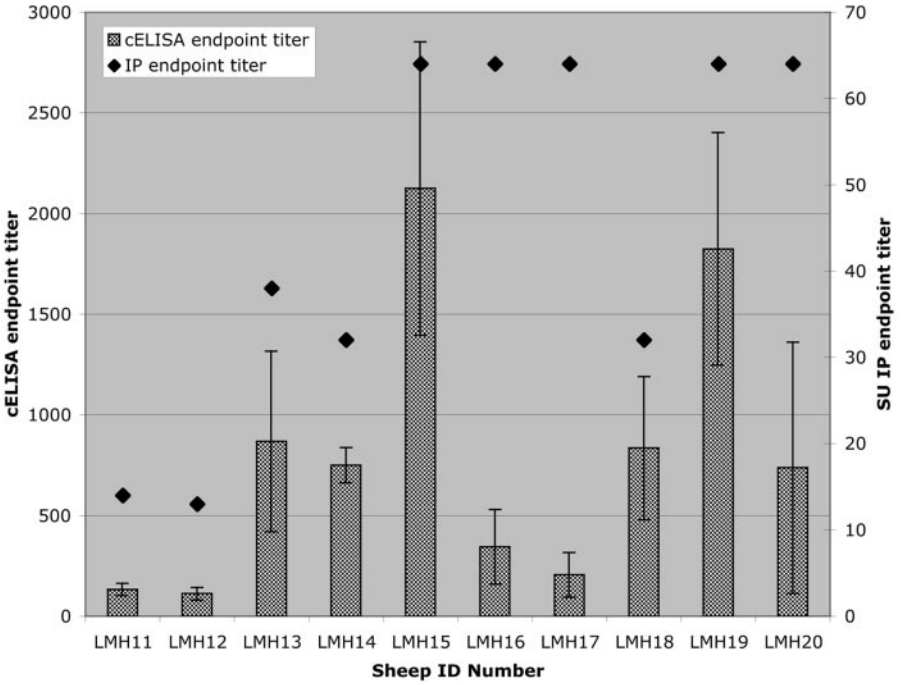

FIG. 3. CAEV cELISA endpoint titers (bar columns) and SU IP endpoint titers (diamonds) are plotted to compare the two assays for detection of anti-SU serum antibody in 10 naturally OPPV-infected sheep. Error bars on the cELISA endpoint titers represent ±1 standard deviation from four replicates. ID, identity.

OPPV antigen as SU. However, since SU IP endpoint titers were 8 to 32 times greater than CA signals and TM90 serum endpoint IP titers were 4 to 32 times greater than CA signals when sera was titrated in 10 out of 10 sheep (Fig. 2), this indicates that the observed B-lymphocyte immunodominance to SU is not due to the increase in specific activity of SU.

Our data also suggest that in some naturally OPPV-infected sheep (2 out of 10 in this study), both SU and TM90 are B-lymphocyte immunodominant. Using immunoprecipitation, the B-lymphocyte-immunodominant viral antigen in the synovial fluid of CAEV experimentally infected goats was SU in one out of five goats, TM90 in one out of five goats, and both SU and TM90 in three out of five goats (7). In another study where Western blot analysis was used, TM90 was determined as the B-lymphocyte-immunodominant antigen in the serum of four out of four goats experimentally infected with CAEV (8). Since Western blot analysis and immunoprecipitation have comparable sensitivities (6), the B-lymphocyte-immunodominant antigen in CAEV-infected goats may be preferentially TM90, whereas in OPPV-infected sheep it is preferentially SU. In general, the prior studies in CAEV along with ours indicate that the immunodominant B-lymphocyte antigen in small ruminant lentiviral infections of mature sheep and goats is SU, TM90, or both SU and TM90.

More specifically, immunodominant continuous B-lymphocyte epitopes have been identified in the C-terminal region of SU and the N-terminal region of TM90 in experimentally CAEV-infected goats using Western blot analysis (1, 2). Also, immunodominant continuous B-cell epitopes in the extreme C terminus of SU have also been identified in goats naturally infected with CAEV using peptide mapping (11). Future studies are planned to precisely map linear and continuous B-lymphocyte epitopes within SU and TM90 of OPPV.

We thank Kathy Pretty On Top-Hötzel for growing and purifying OPPV WLC1. We also thank Nicolas Durfee and Kathy Pretty On Top-Hötzel for performing immunoprecipitations. We thank Emma Karel, Pete Steiner, Duane Chandler, and Steve Parish for their animal care or veterinary care assistance.

REFERENCES

1. **Bertoni, G., C. Hertig, M. L. Zahno, H. R. Vogt, S. Dufour, P. Cordano, E. Peterhans, W. P. Cheevers, P. Sonigo, and G. Pancino.** 2000. B-cell epitopes of the envelope glycoprotein of caprine arthritis-encephalitis virus and antibody response in infected goats. J. Gen. Virol. **81:**2929–2940.
2. **Bertoni, G., M. L. Zahno, R. Zanoni, H. R. Vogt, E. Peterhans, G. Ruff, W. P. Cheevers, P. Sonigo, and G. Pancino.** 1994. Antibody reactivity to the immunodominant epitopes of the caprine arthritis-encephalitis virus gp38 transmembrane protein associates with the development of arthritis. J. Virol. **68:**7139–7147.
3. **Gogolewski, R. P., D. S. Adams, T. C. McGuire, K. L. Banks, and W. P. Cheevers.** 1985. Antigenic cross-reactivity between caprine arthritis-encephalitis, visna and progressive pneumonia viruses involves all virion-associated proteins and glycoproteins. J. Gen. Virol. **66:**1233–1240.
4. **Herrmann, L. M., W. P. Cheevers, K. L. Marshall, T. C. McGuire, M. M. Hutton, G. S. Lewis, and D. P. Knowles.** 2003. Detection of serum antibodies to ovine progressive pneumonia virus in sheep by using a caprine arthritis-encephalitis virus competitive-inhibition enzyme-linked immunosorbent assay. Clin. Diagn. Lab. Immunol. **10:**862–865.
5. **Houwers, D. J., and I. M. Nauta.** 1989. Immunoblot analysis of the antibody response to ovine lentivirus infections. Vet. Microbiol. **19:**127–139.
6. **Johnson, G. C., A. F. Barbet, P. Klevjer-Anderson, and T. C. McGuire.** 1983. Preferential immune response to virion surface glycoproteins by caprine arthritis-encephalitis virus-infected goats. Infect. Immun. **41:**657–665.
7. **Knowles, D., Jr., W. Cheevers, T. McGuire, T. Stem, and J. Gorham.** 1990. Severity of arthritis is predicted by antibody response to gp135 in chronic infection with caprine arthritis-encephalitis virus. J. Virol. **64:**2396–2398.
8. **McGuire, T. C., D. P. Knowles, Jr., W. C. Davis, A. L. Brassfield, T. A. Stem, and W. P. Cheevers.** 1992. Transmembrane protein oligomers of caprine arthritis-encephalitis lentivirus are immunodominant in goats with progressive arthritis. J. Virol. **66:**3247–3250.
9. **Ozyoruk, F., W. P. Cheevers, G. A. Hullinger, T. C. McGuire, M. Hutton, and D. P. Knowles.** 2001. Monoclonal antibodies to conformational epitopes of the surface glycoprotein of caprine arthritis-encephalitis virus: potential application to competitive-inhibition enzyme-linked immunosorbent assay for detecting antibodies in goat sera. Clin. Diagn. Lab. Immunol. **8:**44–51.
10. **Sigurdsson, B.** 1962. Atypically slow infectious diseases, p. 738–753. Extrait du Livre Jubilaire du Dr Ludo van Bogaert, Année 1962. Acta Medica Belgica.
11. **Valas, S., C. Benoit, C. Baudry, G. Perrin, and R. Z. Mamoun.** 2000. Variability and immunogenicity of caprine arthritis-encephalitis virus surface glycoprotein. J. Virol. **74:**6178–6185.